

## 16S profiling and isolation of mucin degraders from human gut microbiota

Musmeci E., Candelieri F., Amaretti A., Raimondi S., Rossi M.\*

\*maddalena.rossi@unimore.it

Department of Life Sciences, University of Modena and Reggio Emilia, Via Campi 103, 40125 Modena, ITALY

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The mucus layer and gut microbiota perform a continuous interaction that contributes to the health status of the host. Mucus harbours gut bacteria and provides carbon and nitrogen sources that support their growth. Mucins are the major building blocks of mucus, consisting of high molecular weight, highly glycosylated proteins. Mucin degraders tightly interact with the host, modulating mucin gene expression, glycosylation, and secretion.

A main task of gut microbiology is to understand the complexity of microbes that colonize the mucus layer, encompassing also the mucin degraders that take advantage of using the carbohydrates decorating mucins as carbon sources, and the protein skeleton as both nitrogen and carbon source. In fact, it has been estimated that 1% of colonic bacteria could degrade mucin using enzymes involved in hydrolysis of the oligosaccharide chains.

In the present study, fecal batch cultures with a medium containing only mucins as fermentable C and N sources were carried out, in order to enrich the bacterial fraction taking advantage of mucin fermentation. Faeces from 5 healthy volunteers were collected and underwent in strict anaerobiosis to three steps of enrichment in mucins medium. The changes of the microbiota composition were monitored by 16S rRNA gene profiling, in order to identify the taxa involved in mucin breakdown and fermentation. At the end of the third passage, different strains have been isolated by plating the enriched cultures in the agarized medium, always with mucin as sole C and N source. Different clones per sample were subjected to RAPD-PCR and clustered into biotypes with similarity level of 75% using the Pearson correlation coefficient. All the bacteria flourishing on mucin, enriched from the 5 diverse fecal samples, belonged to the species *Clostridium disporicum*, *C. tertium*, and *Paraclostridium benzoelyticum*.

This work shed light on the functional relevance of these intestinal species largely diffused in the gut microbiota of healthy humans, that, at best of our knowledge, had never been associated to mucin degradation, and that can exert relevant roles in terms of health maintenance or disease promotion.